LIFE AFTER TRAUMA: SEX DIFFERENCES IN CHILDREN'S CORTISOL STRESS RESPONSE

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Brynne Tynes. Life After Trauma: Sex Differences in Children's Cortisol Stress Response (Dr. Michael Scheeringa and Dr. Gary Dohanich, Neuroscience)

The present study compared cortisol levels at baseline and following exposure to stressors in female and male children 3 to 6 years of age diagnosed with posttraumatic stress disorder (PTSD). Data were collected from 97 subjects diagnosed with PTSD who had experienced a single traumatic event, repeated traumatic events, or Hurricane Katrina. Subjects provided an initial salivary sample, completed a series of stressful laboratory tasks shortly thereafter, and subsequently provided a second salivary sample to measure the cortisol stress response. Data were analyzed via independent samples t-test and multi-factor analysis of variance. Males displayed a slightly higher, but non-significant, baseline cortisol level than females. While this difference was small and non-significant for the single trauma and Hurricane Katrina groups, males in the repeated trauma group exhibited a noticeably higher—yet still non-significant—baseline than females. Analysis of cortisol stress response showed that cortisol levels decreased significantly following exposure to a laboratory-induced stressor. A three-way interaction revealed a significant decrease in cortisol response only in males who had a history of repeated trauma. This decrease was associated with an unusually high baseline cortisol level in these males. These results suggest that, while male and female children with PTSD may not display significant differences in HPA axis activity overall, the type and amount of traumatic events experienced are associated with different cortisol reactivity between the sexes. Further research is needed to fully understand the relationship between type and amount of trauma, sex, and HPA axis activity in children with PTSD.

Acknowledgements

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Introduction

Hypothesis

Measuring hypothalamus-pituitary-adrenal (HPA) axis activity through cortisol levels is one of the main methods for understanding the body's stress response, but research is still relatively limited with young children. The present study aims to compare cortisol stress reactivity of female to male children 3 to 6 years of age exposed to trauma. The first hypothesis states that female subjects with posttraumatic stress disorder (PTSD) will exhibit lower overall resting cortisol levels compared to males with PTSD. The second hypothesis is that females with PTSD will exhibit a smaller cortisol response to lab-induced stress compared to their male counterparts. Possible effects of trauma chronicity will be controlled for through a subgroup analysis of those who experienced a singular traumatic event (e.g., motor vehicle accident) compared to those who experienced repeated trauma (e.g., domestic violence) as well as Hurricane Katrina victims. It is hypothesized that subjects, both male and female, with repeated trauma will show a smaller cortisol response than those with a singular trauma.

HPA Axis

The HPA axis is a major neuroendocrine mechanism that acts to maintain homeostasis in response to stress and contributes to various physiological functions. When the body is exposed to a stressor, a sequence of hormones is released from this system. Corticotropin releasing hormone (CRH) is secreted by the hypothalamus, causing the subsequent release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. This signals the adrenal cortex to release a range of glucocorticoids, with the main glucocorticoid in humans being cortisol (Kudielka & Kirschbaum, 2004). When

this cascade is initiated by stress, cortisol levels elevate in approximately 15 to 30 minutes and typically do not return to normal levels until after the stimulus is discontinued. This activates the fight or flight response, initiating the biological responses such as increased energy and attention necessary to survive a dangerous situation. This response can be accurately quantified by taking salivary cortisol levels, which is a technique that has been used in many studies to measure physiological manifestations of exposure to stressful stimuli (Li et al., 2013).

Cortisol and PTSD

Although some studies have found relationships between cortisol activity and PTSD, the area is still lacking in sufficient conclusive evidence. The findings in many cortisol-PTSD studies have been inconsistent, and none have been conducted in very young children. In a systematic review by Speer and colleagues, a total of 10 studies on the relationship between PTSD and HPA axis activity were examined. Two of the studies found a significant association between PTSD and abnormal diurnal cortisol compared to control groups, five studies reported some correlations between the two variables only at specific times or with experimental manipulation (e.g., after the Trier Social Stress Test), and three studies found no relationship at all (Speer et al., 2019). Although the current study does not examine diurnal cortisol, this example demonstrates the conflicting results yielded by existing research in this subject area and shows the need for further investigation into cortisol levels in individuals, specifically children, with PTSD to clarify the role of HPA axis activity in stress related disorders.

As research on cortisol and PTSD continues to be conducted, several hypotheses about the specific role that cortisol plays in the development and manifestation of the

disorder have developed. Diathesis Stress Theory (DST) suggests that neurobiological irregularities, such as HPA axis activity and cortisol dysregulation, may be present before a traumatic event and subsequently increase the likelihood of developing PTSD following the experience (Lupien et al., 2018). This model has been useful for understanding several psychiatric disorders, including schizophrenia, anxiety, depression, and bipolar disorder (Scheeringa, 2020). Neurotoxic Stress Theory (NST) postulates that neurobiological irregularities are nonexistent prior to the traumatic experience and are instead a symptom that appears after the disorder develops (Mann & Arango, 1992). Generally, toxic agent theories are more relevant when examining infections or diseases caused by airborne substances, living pathogens, or consumption of chemicals, as opposed to psychological trauma (Scheeringa, 2020). In a review paper examining these perspectives in prospective longitudinal studies, Scheeringa found that 19 out of 25 studies showed results in favor of the DST, while only 3 out of 10 studies supported the NST. Clearly, DST is the leading theory in neuroscience and provides the rationale for research on the relationship between cortisol and PTSD, including that of the present study.

Additionally, much literature supports the theory that persons at risk for developing PTSD display reduced levels of cortisol as well as muted circadian rhythms across the course of the day, with levels lower than controls in the morning. A systematic review by Pan et al. (2018) found that morning salivary cortisol levels were consistently lower in subjects with PTSD compared to controls, suggesting a potential vulnerability factor for PTSD.

Another motivation for this line of research is that cortisol in disproportionate amounts is harmful on its own, although the specific impact from high cortisol levels found in PTSD patients is still unclear. Excessive HPA axis activity has several potential consequences, including increased adrenal and pituitary gland size, hyperglycemia, hypertension, declining memory due to damage to the hippocampus, dysfunctional neurotransmitter activity, and more (Stokes, 1995). Learning more about cortisol levels and its effects on the body may prove critical to improving treatment and patient outcomes for those with PTSD and other disorders.

Challenges of Studying Cortisol

Several issues of cortisol can make research more complicated and challenging. One issue is that there are multiple indices of cortisol that may reflect different aspects of regulation, including resting baseline, diurnal pattern, and lab-based controlled stimuli reactions. A second issue is that there is some question as to whether measuring cortisol responses to laboratory stress is comparable to responses to real-life stressors. Studies have shown conflicting results across these issues (Kidd et al., 2014). However, Kidd and colleagues found that cortisol reactivity to lab-induced stress and daily cortisol output were significantly correlated; subjects who demonstrated a greater cortisol response to stress in the laboratory also exhibited an elevated cortisol output throughout the day.

A challenge across methods is that depression seems related to cortisol, and depression is often comorbid with PTSD. Additionally, trauma-exposed individuals who are not diagnosed with PTSD are often included in control groups when studying cortisol levels and PTSD, but may display distinct irregularities from those not exposed to trauma whatsoever. A systematic review and meta-analysis of 47 studies found that, compared to

control subjects who had not been exposed to trauma, morning cortisol levels were lower in subjects with PTSD as well as in those with both major depressive disorder (MDD) and PTSD (Morris et al., 2012). Trauma-exposed subjects with no diagnoses (TE groups) were comparable to controls. Afternoon/evening cortisol levels were lower in subjects with PTSD and in TE groups, but higher in those with both PTSD and MDD. Daily cortisol output was lower in subjects with PTSD and in those with both PTSD and MDD. TE groups were comparable to controls. Therefore, the difference in cortisol levels from morning to evening was greater in subjects with both PTSD and MDD than those with only PTSD. The data suggest that while decreased daily cortisol output may be a specific vulnerability factor for PTSD, increased feedback to the HPA axis may be part of the aftermath of a traumatic experience. The researchers also report that individuals who develop only PTSD and those who develop both PTSD and MDD likely possess both mutual and discrete vulnerability factors that contribute to their condition(s).

Lastly, another challenge is speculation that the type of trauma experienced by victims may result in different outcomes. Namely, interpersonal trauma, which tends to be repeated and chronic, may have more severe impacts that non-interpersonal trauma, which tends to be a single event. The literature in regard to cortisol is limited, but one study of mothers who experienced interpersonal violence showed blunted cortisol reactivity to a laboratory stressor, and so did their 12–48-month-old toddlers (Cordero et al., 2017).

Sex Differences

Sex differences have been observed in research regarding cortisol levels, including healthy females exhibiting lower diurnal cortisol levels than males as well as

less cortisol reactivity to biological challenge tests (Morris et al., 2012). This may help explain sex differences in the prevalence of PTSD.

According to Morris and colleagues, the chances of being diagnosed with PTSD after experiencing traumatic events is twice as high for females, despite the fact that males are more likely to be exposed to trauma. However, the researchers note that this trend may also be due to the type of trauma usually endured by females, which tends to consist of physical and/or sexual abuse.

In a meta-analysis by Meewisse et al. (2007), data showed significantly lower cortisol levels for females with PTSD than without PTSD. This difference was not present in males. The review also demonstrated that subjects who experienced physical or sexual abuse presented with lower cortisol levels than those experiencing other types of traumas (e.g., refugees, war veterans). However, significant overlap between female subjects and those with prior trauma from abuse prevented determination of which factor may have acted to cause the decrease in cortisol. In other words, current understanding of the effects of biological sex on cortisol levels is limited because of the large proportion of females suffering from PTSD due to physical or sexual abuse. The current study will examine both of these subgroups in order to distinguish any relation of the two factors to cortisol levels.

A meta-analysis by Zorn et al. (2017) examining sex differences in cortisol stress reactivity found that females with MDD or an anxiety disorder displayed a smaller cortisol response to stress compared to their healthy counterparts. On the other hand, males with MDD or social anxiety disorder had a larger cortisol response compared to their healthy counterparts. Finally, in contrast, a review paper by Kudielka and

Kirschbaum (2004) concluded that "most psychological stress studies revealed that there are (a) no significant sex differences or (b) higher cortisol responses in young men than in young women after exposure to acute real-life psychological stress (e.g., academic exams) or controlled laboratory stress tasks (e.g., free speech, mental arithmetic, harassment)" (p. 117). The present study aims to assess these issues in very young children exposed to trauma.

There are some speculations as to why sex differences have been present in cortisol activity, including potential interaction between cortisol and sex hormones. One study which observed that males exhibited a larger cortisol response to laboratory stress compared to their female counterparts also revealed negative correlations between progesterone and both ACTH and salivary cortisol reactivity in women, while negative correlations were only found between testosterone levels and cortisol responses in men (Stephens et al., 2016). This suggests that testosterone and progesterone in males and females may have different inhibitory effects on hormone release in the HPA axis. However, since the children in the present study are prepubescent, sex hormones may not play a significant role in cortisol level differences. Another possibility considers the influences of both subjective perception of and biological symptoms of stressors. For instance, males have shown greater cortisol responses to situations involving assessment and accomplishment, while females have shown greater responses to being rejected in social environments (Daughters et al., 2013). Furthermore, situational psychological appraisal of stress may differ between the sexes in response to physiological responses, resulting in varying levels of subjective emotional distress and further cortisol response.

In sum, more research is needed to conclusively determine causes of sex differences in cortisol levels.

Significance

Understanding the biological manifestations of trauma exposure in children may be enlightening for the development of new methods of treatment and promotion of successful development. Three to six years is an age of rapid development, where children are learning to navigate their surroundings, form relationships, and learn socially appropriate behavior. Research on biological differences in manifestations of trauma exposure between sexes can help healthcare providers individualize treatment strategies in order to provide maximally effective care. It may also help develop and advance preventative measures for those at high risk for certain conditions due to irregular cortisol levels. This study may further knowledge of the effects of trauma on young males and females, potentially offering insight into the evaluation and treatment of traumatized children.

Methods

Subjects

Study participants were all between 36 and 83 months of age when initially enrolled in the study and at the time of their most recent trauma. Data were collected from 2003 to 2008 in an NIH-funded R01 study by Dr. Michael Scheeringa, an advisor for this thesis. Three groups of children experiencing either a single traumatic event, repeated trauma, or Hurricane Katrina (a total of 203 subjects, 97 of which were diagnosed with PTSD) were formed in order to investigate the effects of different types of trauma exposure. There was also a control group of children not exposed to trauma.

Children with a head trauma receiving a score of 7 or less on the Glasgow Coma Scale (Teasdale & Jennett, 1974) in the emergency room, intellectual disability, autism spectrum disorder, blindness, deafness, and non-English speaking families were excluded from the study.

Subjects exposed to a single traumatic event (e.g., motor vehicle accidents, witnessing relatives assaulted) were enlisted through newspaper advertisements and a Level I Trauma Center registry. Participants who experienced multiple traumatic events, such as domestic violence over time, were obtained from three main battered women's programs in New Orleans. Children exposed to Hurricane Katrina were recruited through newspaper advertisements. Subjects in the control group who were not exposed to trauma were acquired by contacting neighbors of the trauma-exposed children to create equal sociodemographic factors across participants.

Procedure

On the first visit, caregivers gave their informed consent for the study and participated in a structured diagnostic interview with the Preschool Age Psychiatric Assessment (PAPA; Egger et al., 2006) used to identify and diagnose children with PTSD. Caregivers were compensated $50.

On the second visit, caregivers and children arrived at the lab between 9:00 a.m. and 9:30 a.m. After five to ten minutes of acclimating to the lab, children placed a cotton roll in their mouth for about one minute to provide the first salivary sample, which served as a measure of their baseline cortisol level. The cotton roll was then placed in a Salivette tube and stored in a freezer until it was sent to a lab for salivary cortisol levels to be

analyzed. Caregivers and children then completed a series of stressful tasks outlined below.

For the Memory Stimuli task, children were interviewed using a semi-structured format about a non-traumatic and a traumatic event that they experienced in the past two to six months. In the Videotape Stimuli task, children watched a five minute and 20 second video of clips from theatrical movies consisting of a Mild Distress epoch (a boy being teased), a Happy epoch (children opening gifts on Christmas), and a Trauma epoch (a woman banging on a door and screaming for help at night). A Toy-Take-Away task followed, in which children were given a fun toy called the Jammin' Draw. Forty seconds later, the parent took the toy away and gave them a less exciting one, an immobile human figure.

This series of stimuli took approximately 60 minutes to complete. The child immediately provided a second salivary sample, which served as a measure of salivary cortisol levels in response to the stressful stimuli. It was placed in a Salivette tube and stored in a freezer until it was sent to a lab for salivary cortisol levels to be analyzed. Caregivers were compensated $100.

Data Analysis

The first hypothesis posits that the mean baseline cortisol level (initial sample taken in the lab) of females with the full diagnosis of PTSD will be significantly lower than the mean baseline level of males with PTSD. This will be tested with a t-test.

To further explore the relation of baseline cortisol to type of trauma experience (i.e., single event versus repeated events versus Hurricane Katrina), a two-way, six-group analysis of variance (ANOVA) will be conducted with groups consisting of (1)

female/single event, (2) female/repeated events, (3) female/Hurricane Katrina, (4) male/single event, (5) male/repeated events, and (6) male/Hurricane Katrina.

For the second hypothesis, it is expected that the typical stress response to the lab stimuli will be an increase in cortisol output. My directional hypothesis posits that females with PTSD will demonstrate a smaller increase in cortisol in response to the lab stimuli compared to males. This will be tested with a repeated measures ANOVA.

To further explore the relation of cortisol stress response to type of trauma experience (i.e., single event versus repeated events versus Hurricane Katrina), a three-way, six-group repeated measures ANOVA will be conducted with the same six subject groups described above for the first hypothesis.

Results

Out of 203 total subjects in the cortisol study, 60 males and 37 females were diagnosed with PTSD and their data used to test Hypothesis 1. Two females did not provide a second cortisol level measure and were therefore excluded from the analyses of Hypothesis 2, giving a total of 60 males and 35 females.

Preliminary Analysis

Physiological variables often include outlier values of individuals with extreme responses that can skew means and standard deviations. I examined baseline cortisol values of the full sample of those with PTSD by calculating z scores and applying the Winsorization method (Dixon, 1960). If the z score for the highest cortisol value was >3, this value was omitted and then the z score for the next highest value was calculated. This process identified eight cases as outliers. In order to maintain these individuals in the analyses and maximize power for this small sample, their baseline cortisol values were

replaced with an artificial value that maintained their rank order in the sample. For example, the highest value that was not an outlier was 15.81. The next highest value, 17.52 (z score of 3.13) was assigned a value of 15.9. The next highest value, 18.11, was assigned a value of 16.0, and so on. This process was repeated for the cortisol stress responses, identifying and transforming five outliers.

Hypothesis 1: Baseline Cortisol Levels of Males and Females Diagnosed with PTSD

The mean baseline cortisol level after Winsorization was 7.98 nmol/L (SD 4.07, n = 60) in males and 7.18 nmol/L (SD 3.48, n = 37) in females. Although this shows a slightly higher baseline in males than females, this difference was not statistically significant ($t = 0.983$, $p = 0.328$).

Hypothesis 1: Baseline Cortisol Levels of Males and Females Diagnosed with PTSD in Relation to Type of Trauma

The means and standard deviations for male and female in each trauma group are shown in Table 1. Two-way ANOVA (sex x trauma type) revealed no main effects (sex $F = 1.17$, $p = 0.283$; trauma type $F = 0.326$, $p = 0.723$) or interaction effect ($F = 1.14$, $p = 0.324$). The difference between means of baseline cortisol levels for males and females was extremely small and not statistically significant for either the single trauma group (males 6.80 nmol/L, females 7.15 nmol/L, $p = 0.825$) or Hurricane Katrina group (males 7.82 nmol/L, females 7.53 nmol/L, $p = 0.795$). However, males in the repeated trauma group exhibited a trend toward a higher baseline than females (males 9.40 nmol/L, females 6.44 nmol/L, $p = 0.103$; Figure 1).

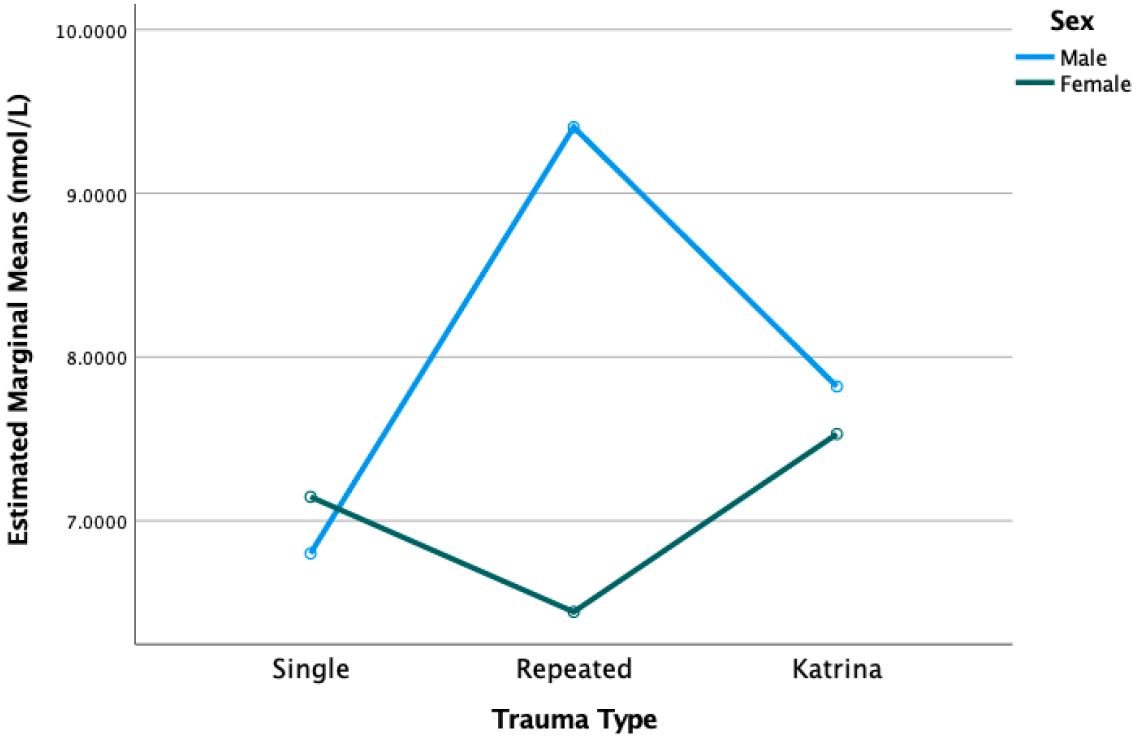
Table 1

Descriptive Statistics for Baseline Cortisol in Relation to Type of Trauma

Sex	Trauma Type	Mean (nmol/L)	Standard Deviation	N
Male	Single	6.80	2.78	11
	Repeated	9.40	4.59	13
	Katrina	7.82	4.17	36
	Total	7.98	4.07	60
Female	Single	7.15	3.97	8
	Repeated	6.44	2.87	9
	Katrina	7.53	3.65	20
	Total	7.18	3.48	37
Total	Single	6.95	3.23	19
	Repeated	8.19	4.17	22
	Katrina	7.72	3.96	56
	Total	7.67	3.86	97

Figure 1

Estimated Marginal Means of Baseline Cortisol in Relation to Type of Trauma

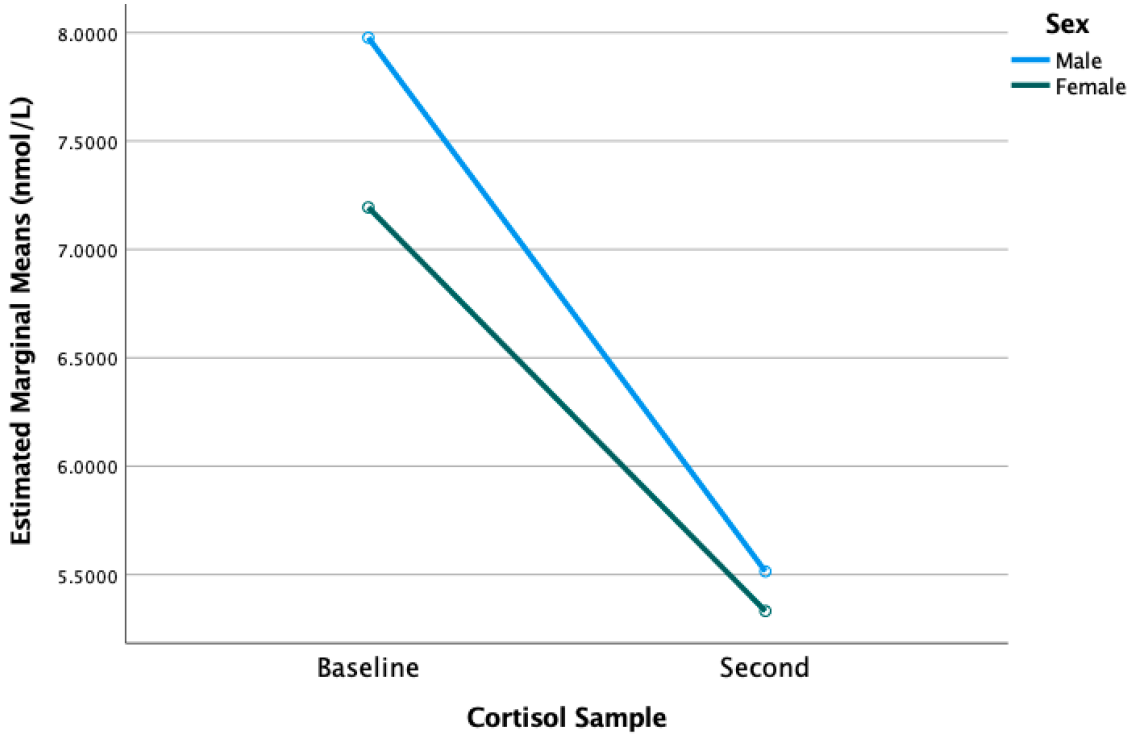


Hypothesis 2: Cortisol Stress Response of Males and Females Diagnosed with PTSD

Overall cortisol levels after completing stressful laboratory tasks decreased significantly from baseline levels (F = 44.64, p = 0.00, Figure 2). Males experienced a larger decline of 2.46 nmol/L from a 7.98 nmol/L baseline cortisol mean to a 5.51 nmol/L second cortisol mean (n = 60), and females had a smaller decrease of 1.86 nmol/L from 7.19 nmol/L baseline to 5.33 nmol/L (n = 35). However, this sex difference in cortisol levels in response to stressful stimuli was not statistically significant (F = 0.860, p = 0.356, Figure 2).

Figure 2

Estimated Marginal Means of Cortisol Stress Response



Hypothesis 2: Cortisol Stress Response of Males and Females Diagnosed with PTSD in Relation to Type of Trauma

Repeated measures ANOVA (cortisol x sex x trauma type) revealed a significant main effect of cortisol before and after exposure to stressful stimuli ($F = 38.69$, $p = 0.00$) and a three-way interaction between cortisol, sex, and trauma type ($F = 3.946$, $p = 0.023$; Table 2). Further analysis of each trauma group revealed that the change in cortisol between males and females was not significant for the single trauma group ($F = 1.922$, $p = 0.184$; Figure 3) or the Hurricane Katrina group ($F = 0.015$, $p = 0.904$; Figure 4). On the other hand, the change in cortisol was significantly greater for males than females in the repeated trauma group ($F = 5.218$, $p = 0.033$; Figure 5).

Table 2

Descriptive Statistics for Cortisol Stress Response in Relation to Type of Trauma

Cortisol Sample	Sex	Trauma Type	Mean (nmol/L)	Standard Deviation	N
Baseline	Male	Single	6.80	2.78	11
		Repeated	9.40	4.59	13
		Katrina	7.82	4.17	36
		Total	7.98	4.07	60
	Female	Single	7.15	3.97	8
		Repeated	6.44	2.87	9
		Katrina	7.59	3.60	18
		Total	7.19	3.45	35
	Total	Single	6.95	3.23	19
		Repeated	8.19	4.17	22
		Katrina	7.74	3.95	54
		Total	7.69	3.86	95
Second	Male	Single	5.55	2.07	11
		Repeated	5.39	2.26	13
		Katrina	5.55	2.61	36
		Total	5.51	2.41	60
	Female	Single	4.40	1.82	8
		Repeated	5.99	3.04	9
		Katrina	5.42	2.40	18
		Total	5.33	2.46	35
	Total	Single	5.07	2.00	19
		Repeated	5.64	2.55	22
		Katrina	5.50	2.52	54
		Total	5.45	2.42	95

Figure 3

Estimated Marginal Means of Cortisol Stress Response for Single Trauma Group

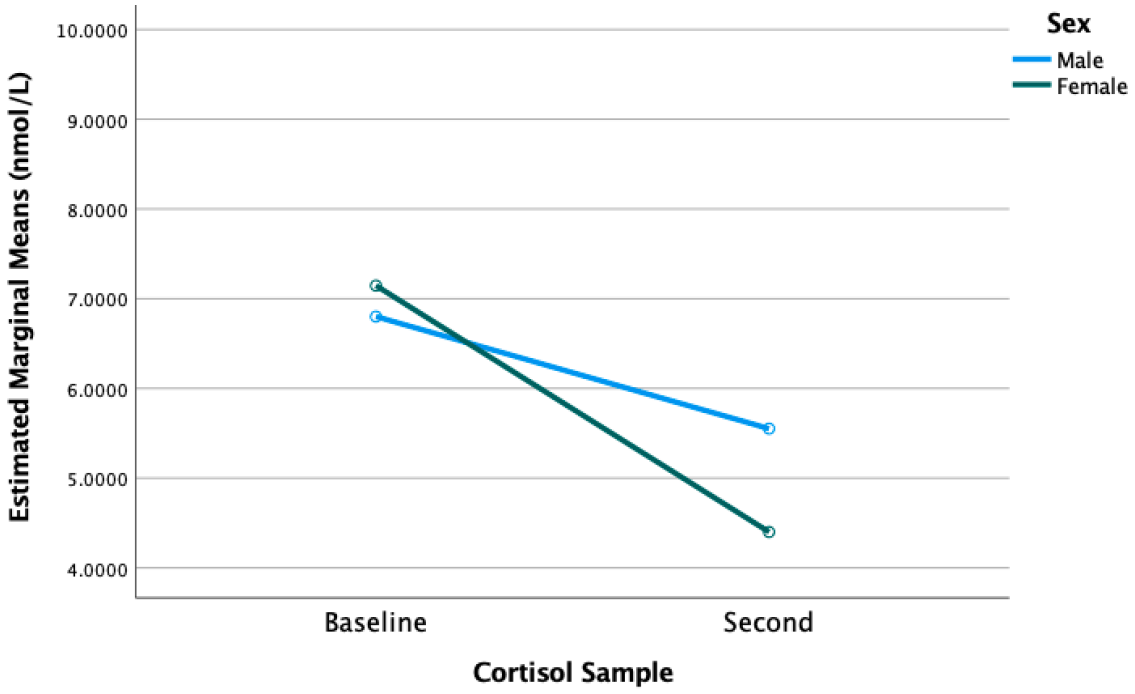


Figure 4

Estimated Marginal Means of Cortisol Stress Response for Hurricane Katrina Group

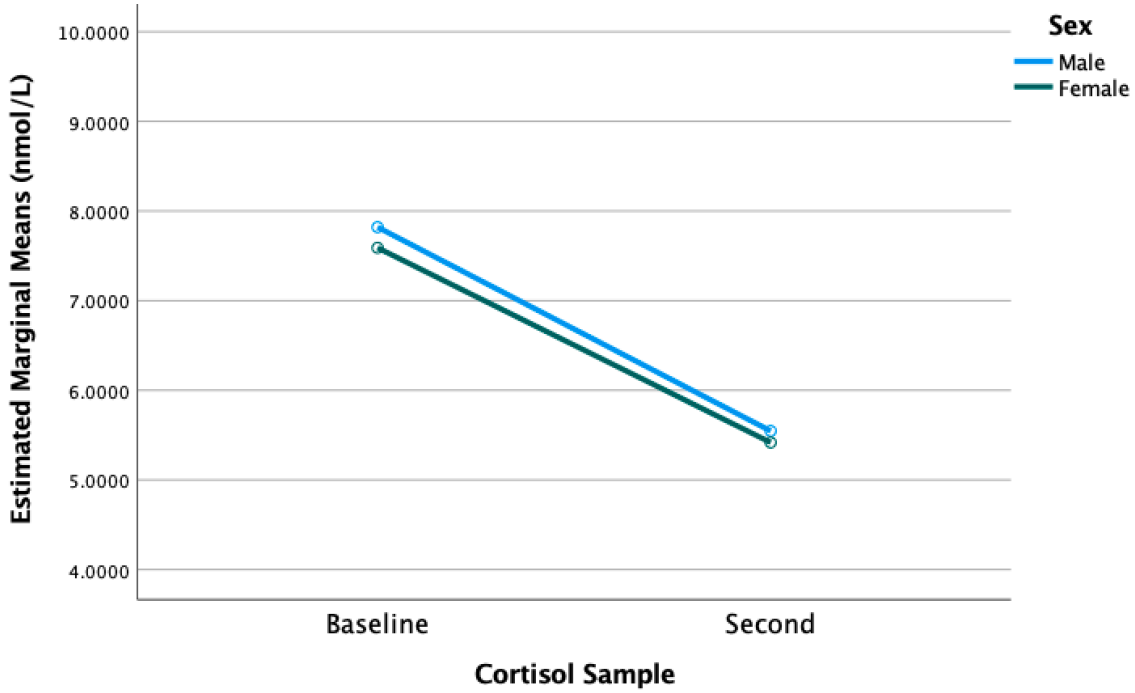
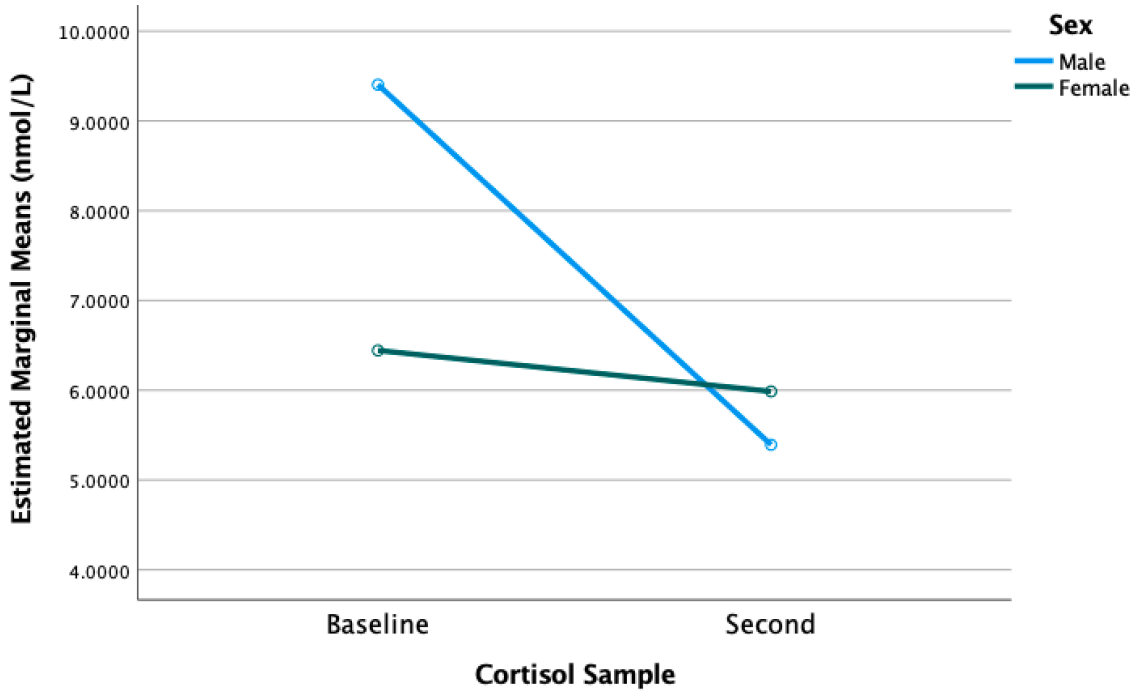


Figure 5

Estimated Marginal Means of Cortisol Stress Response for Repeated Trauma Group



Post Hoc Analysis of Repeated Trauma Group

To explore the difference found in the repeated trauma group, further investigation was conducted. The mean number of types of traumatic events that each subject experienced was identical for males and females, at 1.8 and 1.8 events, respectively, and the medians were equal at two events for both sexes. On the other hand, the total number of occurrences of traumatic events per subject differed dramatically. On average, males experienced 138.8 total occurrences (with a median of 11), while females experienced 10.6 (with a median of 7).

No outstanding differences were observed between males and females in the types of traumas experienced by the repeated trauma group (Table 3). All subjects in this group, regardless of sex, experienced or witnessed domestic violence. No females and one male endured sexual abuse.

Table 3

Types of Traumas Experienced by Males and Females in Repeated Trauma Group

Trauma Type	Male		Female		Total	
	Yes	No	Yes	No	Yes	No
MVA	5	20	3	10	8	30
Animal Attack	0	25	2	11	2	36
Manmade	1	24	1	12	2	36
Natural Disaster	1	24	1	12	2	36
Domestic Violence	25	0	13	0	38	0
Physical Abuse	1	24	1	12	2	36
Sexual Abuse	1	24	0	13	1	37
Burn	6	19	0	13	6	32
Near Drown	1	24	0	13	1	37
Medical	2	23	0	13	2	36
Kidnapping	0	25	1	12	1	37
Other	1	24	1	12	2	36

Discussion

Interpretation of Results

Although males displayed a slightly higher baseline cortisol level than females, which aligns with my original hypothesis that female subjects with PTSD would exhibit lower overall resting cortisol levels compared to males with PTSD, this difference was not statistically significant. Interestingly, while the difference between average male and female baseline cortisol levels was extremely small and not statistically significant for both the single trauma and Hurricane Katrina groups, males in the repeated trauma group exhibited a noticeably higher—yet still non-significant—baseline than females. These results indicate that children with PTSD did not display significant differences between

sexes in baseline cortisol levels overall. However, the current results suggested a sex difference in resting cortisol when comparing male and female children experiencing specific types of trauma. Unfortunately, this sex difference failed to reach statistical significance, which may have been revealed by a larger sample size that provided more statistical power.

Analysis of the cortisol stress response between sexes showed a significant decrease from baseline cortisol measure to second cortisol measure in both males and females overall, which opposes the anticipated result of an increase in cortisol after enduring stressful conditions. Females exhibited a smaller decrease in cortisol than males, consistent with my original hypothesis that females with PTSD would exhibit a smaller cortisol response to laboratory-induced stressors (albeit in the opposite direction) compared to their male counterparts, though this difference was not statistically significant. Comparing this reaction to the type of trauma previously experienced by subjects revealed that the difference in cortisol stress response between males and females was statistically non-significant for the single trauma group as well as the Hurricane Katrina group, but statistically significant for the repeated trauma group. Within the repeated trauma group, females exhibited a lower cortisol baseline, as well as a weaker decrease in cortisol stress response, while males exhibited a distinguishably higher cortisol baseline with a much larger reduction in cortisol levels following exposure to stressful stimuli. This result does not coincide with my original hypothesis, which stated that subjects, both male and female, with repeated trauma would show a smaller cortisol response than those with a singular trauma. Again, these results show that while male and female children with PTSD may not exhibit significant differences in cortisol

stress response overall, certain types of past traumatic experiences may be associated with differential reactions to future stress that interact with the biological sex of the child. Further examination of the repeated trauma group showed that males experienced considerably more occurrences of traumatic events than females on average, which may have played a moderating role in the divergence of the repeated trauma group in both baseline and reactionary cortisol measures.

Context of Relevant Literature and Existing Research

Stressful events initiate release of cortisol by the HPA axis, elevating cortisol levels in the body and contributing to the fight or flight response (Li et al., 2013). The data in the present study demonstrated the opposite relationship, showing a decline in salivary cortisol levels following laboratory-induced stress. This may indicate that HPA axis activity is different in individuals with PTSD, resulting in a decrease in cortisol rather than an increase in response to stress. These irregularities may be present before the traumatic event(s) as suggested by the Diathesis Stress Theory (DST), which proposes that existing abnormalities predispose some trauma victims to the development of PTSD symptomology (Lupien et al., 2018). Additionally, the results of the present study support the conclusion of Kudielka and Kirschbaum (2004) that in most cases, cortisol studies show either no significant differences between the sexes or a larger cortisol response in males than females following real world or laboratory-induced stress.

According to past research, males are more likely than females to be exposed to trauma (Morris et al., 2012). This finding was reflected in the present analysis, which revealed a significantly higher number of traumatic events experienced by males as compared to females in the repeated trauma group; this may have been responsible for the

significant discrepancy in cortisol stress response observed between sexes in this group. Also, lack of divergence in type of trauma between the sexes may suggest that it is not a moderating factor of the sex differences in HPA axis activity observed in the present study, as has been proposed by other researchers (Meewisse et al., 2007). Finally, the significant findings regarding only the repeated trauma group may be due, in part, to the more severe impact of the interpersonal nature of repeated trauma types suggested by previous research (Cordero et al., 2017).

Limitations and Future Research

A possible limitation of this study includes the time of day when cortisol measures were taken, as cortisol levels fluctuate throughout the day and may affect baseline cortisol levels as well as the possible range of stress responses observed in the subjects. Taking cortisol measures in the evening may have yielded different results than the present study, which collected cortisol exclusively in the morning hours. However, gathering cortisol data during the same time window for all subjects was a method designed by experimenters to minimize any potential error due to this variable. Measuring cortisol intermittently over a longer period of time (e.g., 24 hours) may have also revealed different reactions to stress or significant patterns in cortisol levels between groups.

Other potential limitations include that this study used laboratory-induced stress to stimulate a cortisol response, which may or may not be comparable to the reaction produced by real world stressors and might limit the generalizability of the results. Furthermore, comorbidity of PTSD with MDD was not considered in the present study, which may have an influence on cortisol levels and stress reactivity and should be

reflected in future studies. Notably, only one female and two males endured sexual or physical abuse, and results from this study may not be generalizable to a sexually abused population which typically includes more females than males (Morris et al., 2012). Race, socioeconomic status, family/living conditions, or other factors not accounted for in this study may influence cortisol levels in children as well, although recruitment techniques were designed to maintain as consistent sociodemographic conditions across participants as possible. Additionally, as previously mentioned, a larger sample size may have allowed for more accurate in-depth analysis and investigation of cortisol differences between subgroups.

Future research on type and amount of trauma experienced by children with PTSD in relation to sex may further understanding of the relationship between these factors, and, in turn, to cortisol stress response. In the present study, the repeated trauma group deviated from other groups—additional investigation into individuals enduring traumatic events over an extended period of time could reveal more about the implications of trauma in these children. Comprehending these biological differences in manifestations of trauma exposure in children may provide insight into potential treatment methods and strategies to promote successful development in this vulnerable population.

Conclusion

The objective of this study was to compare baseline cortisol levels and cortisol stress responses between male and female children diagnosed with PTSD. Results suggest that, although males and females may not display significant differences in cortisol levels or stress reactivity overall, the type and amount of trauma exposure is

associated with different HPA axis activity between the sexes. Additional research into children with PTSD experiencing repeated trauma over time is imperative to fully understand the difference in biological manifestations of trauma between males and females.

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